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13. ABSTRACT ***(Maximum 200 Words)***

It is well known that many breast tumors associate with estrogen and the presence of catecholestrogens in breast tumors cause changes in the DNA which may lead to uncontrolled cell growth. Catecholestrogens are broken down by an enzyme called catechol-O-methyltransferase (COMT). COMT is known to be elevated in malignant breast tumors, and abnormal COMT genetics have recently been found in individuals with breast cancer. We have developed [^{18}F]Ro41-0960, the first radiotracer for visualizing COMT with positron emission tomography (PET). The hypothesis that [^{18}F]Ro41-0960 can map COMT *in* vivo was demonstrated in baboon and mouse. A complementary *ex vivo* approach in which we correlated the radiotracer uptake with COMT activities in rodents further demonstrated the ability of [^{18}F]Ro41-0960 in mapping COMT activity *in vivo*. Recently, we have adapted our COMT enzyme assay method and performed studies in breast tumor tissue samples from cancer patients undergoing surgery. COMT activities in normal and abnormal human breast tissues from the same patient were compared. Though COMT activities varied among subjects, preliminary results showed elevated COMT activities in the breast tumor tissues of all patients studied; the difference can be as high as 26 fold increase. This novel approach to an extremely important medical problem goes beyond diagnosis in that it seeks to delineate the fundamental biochemical properties and molecular signatures of tumor cells. PET imaging studies on breast cancer patients are underway.

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FOREWORD

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__ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

__ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

N/A In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

6/28/00

PI - Signature Date

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INTRODUCTION

Malignant cells grow out of control because the normal biochemical processes regulating growth are disrupted. A knowledge of the biochemical processes which are disrupted is a key to understanding how cancer develops, to detect it at an early stage and to develop and monitor treatment. One key area is to understand estrogen metabolism in breast tumors. It is well known that many breast tumors depend on estrogen and there is now evidence the presence of estrogen metabolites (called catecholestrogens) in breast tumors cause changes in the DNA which may lead to uncontrolled cell growth. Catecholestrogens are broken down by an enzyme called catechol-O-methyltransferase (COMT). COMT is known to be elevated in malignant breast tumors and abnormal COMT genetics have recently been found in individuals with breast cancer. We recently developed [^{18}F]Ro41-0960, a new radiotracer for visualizing COMT with positron emission tomography (PET). Here we propose to investigate whether COMT activity as visualized with PET can serve as a molecular signature for breast cancer and in this way to aid in the diagnosis of breast cancer. To accomplish this we have the following two specific aims: (1) to perform studies in breast tumor tissue samples from cancer patients undergoing surgery to determine whether the binding of [^{18}F]Ro41-0960 correlates with the activity of COMT; and (2) to determine feasibility of PET imaging COMT with [^{18}F]Ro 41-0960 in human breast cancer patients with grade III breast carcinomas. We predict that the uptake of [^{18}F]Ro41-0960 in breast tumor tissue will be substantially greater than that for normal surrounding tissue, and that in breast cancer patients tumors will be visualized with PET owing to significantly greater tracer uptake in tumor relative to surrounding normal tissue. This novel approach to an extremely important medical problem goes beyond diagnosis in that it seeks to delineate the fundamental biochemical properties and molecular signatures of tumor cells. If successful this new method would reduce the need for unnecessary breast biopsies and enhance the staging capabilities of current diagnostic procedures. Additionally, the discovery of the biochemical processes involved in the deregulation of cell growth may suggest new opportunities for treatment in the future.

Technical Objectives

Specific Aim 1.
In vitro studies in breast tumor tissue samples from cancer patients undergoing surgery: We will determine whether the degree of binding of [^{18}F]Ro41-0960 is sensitive to and reflects the activity of COMT. The correlation between the radiotracer bindings and COMT activities will serve as a model prior to human PET studies.
Specific Aim 2.
Human PET imaging: We will determine feasibility of PET imaging of COMT with [^{18}F]Ro 41-0960 in human breast cancer patients with grade III and IV breast carcinomas. As part of these studies, we will correlate [^{18}F]Ro41-0960 binding with COMT levels obtained by analysis of biopsied tissue. For this specific aim we will obtain an IND for using [^{18}F]Ro41-0960 in humans.

PROGRESS REPORT

Specific Aim (1): Correlate [^{18}F]Ro41-0960 binding with COMT activities in normal and abnormal tissues from the same patient suffering with breast cancer.

We have demonstrated in baboon and mouse that the binding of [^{18}F]Ro41-0960 to COMT sites in periphery is saturable and sensitive to COMT inhibition [Ding, et al.,1996; Ding, et al.,1998; Ding, et al.,1997]. An *ex vivo* approach in which we correlated the radiotracer uptake with COMT activities in rodents further demonstrated the ability of [^{18}F]Ro41-0960 in mapping COMT activity *in vivo* [Ding, et al.,1999]. Recently, we have adapted our COMT enzyme assay method and performed studies in breast tumor tissue samples from cancer patients undergoing surgery. COMT activities in normal and abnormal human breast tissues from the same patient were compared. This will serve as a model prior to human studies.

Assay for COMT Activity: Each tissue sample was weighed, minced and homogenized using a Polytron (setting 7) in 1-5 mL* of ice cold 0.01 M Tris buffer (pH 7.4) containing 12 mM dithiothreitol and 10% glycerol. This was followed by sonication for 15 seconds and then centrifuged at 14,000 xg (Eppendorf) for 30 minutes at 4^{0}C. Aliquots of the supernatant from each sample thus prepared were assayed for COMT activity.

COMT activity was determined by measuring the amount of radioactive metanephrine formed from epinephrine and S-adenosyl-(methyl-^{3}H) methionine (Assicot, et al., 1977). Portions of enzyme preparations from breast tissues (see below for protein assay) were incubated at 37^{0}C for 20 min with 10 μmole of sodium phosphate buffer (pH 7.8), 1 μmole of $MgCl_2$, 0.15 μmole of epinephrine and 3.6 nmole of S-adenosyl-(methyl-^{3}H) methionine in a final volume of 0.15 mL. The enzyme reaction was quenched with 0.5 mL of 0.13 M borate buffer (pH 10). For each tissue sample, four enzyme preparations were carried out: (1) mixture containing enzyme homogenate with and without catechol substrate; and (2) with and without COMT inhibitor (10 μL of Ro41-0960 solution (1 mg/mL) in ethanol). The metanephrine thus formed was then extracted into 5 mL of ethyl acetate. After shaking for 5 min on a Aliquot Mixer (Ames), the tubes were centrifuged at 1000 rpm for 5 min and returned to an ice bath. A portion of the organic layer (2 mL) was then added to 5 mL of scintillation fluid and assayed for radioactivity. COMT activity was expressed as pmole of radioactive metanephrine formed per 20 min per mg of cytosol protein.

Assay for Protein: Protein concentration will be determined according to the method of Lowry *et al.* [Lowry, et al.,1951] using crystalline bovine serum albumin as a standard.

*The amount of Tris buffer used in the tissue homogenate preparation was dependent on the size of the tissue sample; too dilute enzyme preparations wouldn't give reliable data. In the past, we often received tissue samples that were too small to work with. Ideally, a 100-300 mg tissue sample would allow us to carry out duplicate experiments, and tissue samples larger than 50 mg would give a reliable single data point.

Summary of the Results for Specific Aim (1):

COMT activities in normal and abnormal human breast tissues from the same patient were compared. Protein assay was used to normalize the amount of protein used in each experiment, and COMT activity was expressed as pmole of radioactive metanephrine formed per 20 min per mg of cytosol protein. Though COMT activities varied among subjects, preliminary results showed elevated COMT activities in the breast tumor tissues of all patients studied; the difference can be as high as 26 fold increase (Table I). This could be a potential signal to noise ratio for the future PET studies with [^{18}F]Ro41-0960 in breast cancer patients. The most important finding is that these data were consistent with the pathology reports obtained for each patient.

Table I. Catechol-O-methyltransferase Activity in Normal and Abnormal Tissues from the Same Patient

Date of Surgery	Patient Code	Normal Breast Tissue	Breast Tumor Tissue
7/31/98	JB	0.024	1.24
8/7/98	VA	0.028	0.74
8/11/98	RL	0.40	0.40
8/13/98	JB	0.55	0.88
8/13/98	RD	0.11	0.25
1/12/99	GI	0.198	0.66
1/12/99	DO	0.563	2.54
3/09/99	SA	0.28	2.5

COMT activity is expressed as pmole of radioactive metanephrine formed per 20 min per mg of cytosol protein. [Assicot, et al.,1977; Ding, et al.,1999]

Specific Aim (2): Initiate studies in human subjects (female normal controls and females with breast carcinomas). This specific aim has two parts: (2a) preparation of an IND for approval to do PET scanning in humans with [^{18}F]Ro41-0960 and (2b) PET scanning in human breast cancer patients.

2a. IND Application

On February 1998, we submitted an IND to apply for permission to carry out human PET studies with [^{18}F]Ro41-0960 and have been in communication with the FDA since then. Though the amount of [^{18}F]Ro41-0960 which we will use in humans is very small (< 5 micrograms total dose/PET study), the FDA requires a toxicology study performed by a GLP laboratory before they can approve the use of the tracer in PET studies. Our IND application to carry out human PET studies was finally approved by the FDA on October 1, 1999 (attached in the Appendices). This was accomplished by (1) providing preliminary toxicology of Ro41-0960; (2) purchasing an acute toxicity study from Covance Laboratory (Princeton, NJ) in rats as suggested in communications with the FDA.

(1). Preliminary toxicology of Ro41-0960: We have obtained from Hoffmann-La Roche Company the preliminary toxicology data of both COMT inhibitors Ro41-0960 and Ro40-7592 (tolcapone, used for treatment of Parkinson's disease). Both compounds have the same maximum tolerated dose of 312 mg/kg P.O and also the same lethal dose, namely 625 mg/kg P.O. In addition, we have carried out PET studies in baboons with i.v. injection of Ro41-0960 at doses of 0.025 mg/kg-2mg/kg and no hemodynamic effects were observed. Human PET imaging studies require the injection of about 5 mCi [^{18}F]Ro41-0960 for PET imaging. An i.v. dose of 5 mCi [^{18}F]Ro41-0960 with a specific activity of 0.7 Ci/μmol would only contain less than 5 μg of Ro41-0960. Radiation dosimetry which has already been estimated from whole body PET scans in baboons was included as part of the IND application.

(2). Toxicology study: In consultation with the FDA, the following toxicology tests of Ro41-0960 were conducted in order to obtain IND for using [^{18}F]Ro41-0960 in breast cancer patients:

Objective: to evaluate the acute toxicity of Ro41-0960 when administered as a single intravenous injection in rats.
Species: rats.
Gender: female, since we will only be imaging female breast cancer patients.
Type of study: single dose acute toxicity test.
Dose: maximum permitted by the solubility of Ro41-0960. The solubility of Ro41-0960 is about 0.6 mg/ml and the administration was by a single intravenous injection into a tail vein at a dose volume of 1.0 mL/kg. This is 0.6 mg/kg or 7,000 times greater than the maximum dose which would be given to a breast cancer patient for a PET scan.
Groups:
(a) Control Group: Animals will be sacrificed at Day 3 (n=5) and Day 15 (n=5) after i.v. administration of the vehicle control material mixture (5% ethanol in saline)
(b) Test Group: Animals will be sacrificed at Day 3 (n=5) and Day 15 (n=5) after i.v. administration of the test material (0.6 mg/mL of Ro41-0960 in 5% ethanol in saline).

Tests:
Clinical observations, Clinical Pathology (including hematology, coagulation, clinical chemistry), and Histopathology (on heart, kidneys, and liver) were conducted and evaluated.

Summary of the Results for Specific Aim (2a):

The acute toxicity of Ro41-0960 was evaluated in female rats when the test material was administered as a single intravenous injection. All animals appeared normal throughout the study. There were no significant differences in body weights or body weight gains between the control and test groups. Administration of the test material had no related effects on clinical pathology test results. Administration of the control or test materials did not result in any macroscopic or microscopic lesions or findings at necropsy that were directly related to the test or control materials. The No Observance Effect Level (NOEL) for Ro41-0960 given as a single intravenous injection to female rats was 0.6 mg/kg.

IRB (Institution Review Board) approval:

As a part of requirements for human studies, we have also obtained approvals from both the IRBs of Brookhaven National Laboratory (BNL) and our collaborating institute (Stony Brook University, Hospital & Medical Center) (attached in the Appendices). Breast cancer patients will be recruited from Stony Brook Hospital, and PET scans with [^{18}F]Ro41-0960 will be conducted on patients at BNL.

KEY RESEARCH ACCOMPLISHMENTS

(1). COMT activities in normal and abnormal human breast tissues from the same patient were compared. Preliminary results showed elevated COMT activities in the breast tumor tissues of all patients studied; the difference can be as high as 26 fold increase. This will serve as a model prior to human studies.

(2). Our IND application to carry out human PET studies was approved by the FDA on October 1, 1999.

(3). We have also obtained approvals from both the IRBs of Brookhaven National Laboratory (BNL) and our collaborating institute (Stony Brook University, Hospital & Medical Center) to carry out PET studies using [^{18}F]Ro41-0960 on breast cancer patients.

REPORTABLE OUTCOMES

Manuscripts:

Ding Y-S, Logan JS, Gatley SJ, Fowler JS, Volkow ND: PET Studies of Peripheral Catechol-O-methyltransferase in Non-human Primates Using [^{18}F]Ro41-0960. J. Neural. Transm., 105, 1199-1211 (1998).

Ding, Y-S. Fluorine-18 Labeled Biomolecules for PET Studies in the Neurosciences. J. Fluorine Chem. 101, 291-295 (2000).

Abstracts:

Y.-S. Ding, Bea Pyatt, J.S. Fowler, N.D. Volkow, Naji Abumrad, D. DeRisi. A Potential Use of [^{18}F]Ro41-0960, a Selective COMT Inhibitor, for PET Imaging of Estrogen Metabolism in Breast Cancer. 46th Annual Meeting of the Society of Nuclear Medicine Los Angeles, CA, June 6-10, 1999

Oral presentations (Invited lectures):

International Conference on Fluorine Chemistry (ICFC), Tokyo, Japan, May 9-11, 1999. *(Fluorine-18 Labeled Biomolecules for PET Studies in the Neurosciences)*

The 8th International Conference: Peace through Mind/Brain Science, Hamamatsu, Japan, Feb. 2-4, 2000. *(The Neurochemistry of Substance Abuse)*

St. John University at New York, Pharmacology Department, Feb. 29, 2000. *(PET Studies in Neuroscience)*

National Institutes of Health (NIH) and National Institutes of Mental Health (NIMH), Bethesda, Maryland, April 17-18, 2000. *(Highlights of PET Studies in Neuroscience at Brookhaven)*

The 15th Winter Fluorine Conference, St. Petersburg Beach, Florida, January 14-19, 2001. *(PET Studies of Fluorinated Radiopharmaceuticals)*

The Cancer Institute of Long Island at Stony Brook, School of Medicine, State University of New York at Stony Brook, New York, June 14, 2000. *(Novel Approach to Image Estrogen Metabolism in Breast Cancer Using PET)*

CONCLUSIONS

Our ability to assay COMT activities in human breast tissues sets the stage for us to examine the role of COMT in breast cancer, and will allow us to correlate between the radiotracer bindings and tissue COMT activities in the future PET imaging studies with [^{18}F]Ro41-0960. Preliminary results showing elevated COMT activities in the breast tumor tissues of all patients studied support our hypothesis that elevated uptake of [^{18}F]Ro41-0960 would be observed in breast cancer patients.

This novel approach to an extremely important medical problem goes beyond diagnosis in that it seeks to delineate the fundamental biochemical properties and molecular signatures of tumor cells. The benefit of this new knowledge would lead to a better understanding of the biochemistry of human breast cancer, suggest new therapies based on the tumor's molecular profile, and enhance the detecting and staging capabilities of current diagnostic procedures.

REFERENCES

Assicot M, Contesso G,Bohuon C (1977): Catechol-*O*-methyltransferase in human breast cancers. Europ. J. Cancer 13:961-966.

Ding Y-S, Fowler J F, Volkow N D, Abumrad N,Derisi D (1999): A potential use of [^{18}F]Ro41-0960, a selective COMT inhibitor, for PET imaging of estrogen metabolism in breast cancer. J. Nucl. Med. 40:100p.

Ding Y-S, Gatley S J, Fowler J S, Chen R, Volkow N D, Logan J, Shea C E, Sugano Y,Koomen J (1996): Mapping Catechol-*O*-methyltransferase in vivo: Initial Studies with [^{18}F]Ro41-0960. *Life Sciences* 58:195-208.

Ding Y-S, Logan J, Gatley S J, Fowler J S,Volkow N D (1998): PET studies of peripheral catechol-O-methyltransferase in non-human primates using [^{18}F]Ro41-0960. J. Neural. Transm. 105:1199-1211.

Ding Y-S, Sugano Y, Koomen J, Fowler J S, Aggarwal D, Ferrieri R,Schlyer D (1997): Synthesis of [^{18}F]RO41-0960, A Potent Catechol-O-Methyltransferase Inhibitor, for PET Studies. J. Label. Cmpd. Radiopharm. 39:303-318.

Lowry O H, Rosebrough N J, Farr A L,Randall R J (1951): Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265.

Memorandum

To: Nora Volkow, M.D. (Brookhaven National Laboratory)

CC: Yu-shin Ding, Ph.D.; Joanna S. Fowler, Ph D.

From: Kaye Cho, Pharm.D. (DMIRDP/CDER/FDA)

Date: 10/1/99

Re: Status of IND 55,193

This memorandum is written to notify the sponsor that the sponsor has adequately addressed the issues raised during the teleconference on March 6, 1998, and that the sponsor can proceed with their human study under this IND.

BROOKHAVEN NATIONAL LABORATORY
MEMORANDUM

DATE: May 30, 2000

TO: Yu-Shin Ding

FROM: M.C. Bogosian, Chairman, Institutional Review Board (IRB)

SUBJECT: **IRB Protocol 324** "PET Studies of Catechol-O-methyltransferase (COMT) with [^{18}F]Ro41-0960"

At the 05/23/00 IRB meeting, the request to restart the above protocol was approved.

Approval by the CRC Manager is required before work may begin under this protocol.

This protocol is approved until 11/02/00. This approval is given only for the protocol submitted; any changes must be approved by the IRB prior to being implemented.

You should be aware that all research outlined in this protocol must be carried out under approved Experimental Safety Review(s) (ESR) and that this application must contain the same information as that listed in the approved ESR(s). You must be aware that it is your responsibility to ensure that all individuals working on this protocol have been listed on an appropriate ESR and that their training is up to date.

Investigators must report any unanticipated problems promptly to the IRB.

IRB

M.C. Bogosian
B.D. Breitenstein
S. Mendelsohn
H.L. Atkins
A.Z. Diaz
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MCB:dm
IRB Form 010: Approved 08/04/98; Revised 06/01/99

STONY BROOK
STATE UNIVERSITY OF NEW YORK

Office of the Vice President for Research
Research Informatics and Compliance

TO: **Margaret Kemeny**

FROM: **Judy Matuk, University Coordinator for Research Compliance**

SUBJECT: **Approval for Research Involving Human Subjects (APP)**

DATE: **01/20/2000**

The project referenced below was reviewed by the Committee on Research Involving Human Subjects (CORIHS: Assurance #M1036-01) and approved on: **1/14/00** . Attached is a copy of the approved consent form and the human subjects application with the endorsement of CORIHS and the Institution. This approval is valid for one year.

Federal regulations require that:

1. **all research involving human subjects be reviewed at least once annually. You will be sent an application for renewal of CORIHS approval two months prior to the anniversary date.**
2. **any modifications in the project as approved by this Committee involving changes in the selection of subjects, the means for obtaining informed consent, the wording of the approved consent form(s), or in the risk to subjects be sent to the Committee for review and approval prior to initiation.**
3. **the Principal Investigator must keep consent forms with patient/subject signatures in a locked file to ensure confidentiality.**

This approval is subject to recall if at any time the conditions and requirements of the CORIHS are not met. This is for the protection of all parties: the subjects, the investigators, the University and CORIHS.

Description of Study:

Project ID: 19993755

Approval Period: 1/14/00 - 1/13/01

Project Title: PET Imaging Of Breast Cancer Using (18F) Ro41-0960

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